LEG AUTOTOMY AND REGENERATION IN A POPULATION OF *DIDYMURIA VIOLESCENS* (LEACH) (PHASMATODEA: PHASMATIDAE) IN NEW SOUTH WALES, AUSTRALIA

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Abstract

A population of the spurlegged phasmatid, *Didymuria violescens* (Leach), was surveyed in New South Wales, Australia, in order to determine rates of leg autotomy and regeneration in natural environments. Autotomy was common during all instars, and rates of regeneration ranged from ~10% in nymphs to ~25% in adults. Autotomy and/or regeneration also appeared to negatively affect survivorship.

Introduction

All phasmids have the ability to shed and regenerate legs (Bordage 1905, Ramme 1931, Carlberg 1986, Brock 1999). There are two advantages in shedding legs (autotomy): predation avoidance and moulting. If a predator grabs a leg instead of the body, the animal can shed the leg and survive. In addition, legs can be lost to a fouled moult. Phasmids moult upside down and separate themselves from their old exoskeleton with elaborate twisting and turning manoeuvres. During moulting, the long, thin legs, so effective at conferring crypsis, can become entangled in the old cuticle and must be shed to survive.

Complete replacement of a lost leg requires three consecutive instars, each moult producing a successively larger leg. The first moult after leg loss produces a leg about one-quarter the normal size, the second moult produces a leg about half the normal size, and the third moult produces a leg that is ~10% smaller than a non-regenerated leg (see Figure 1). Regenerated legs never attain their full proportions, even with several moults remaining in their life cycle (Ramme 1931, Carlberg 1986, 1992, T. Maginnis personal observation). Hatchlings which lose a leg during the first instar do not produce regenerated legs until the third instar and, with the exception of the instar before maturity, nymphs always commence the regeneration process (T. Maginnis personal observation).

As in many other species with regenerative capacities, little is known about how often phasmids lose and regenerate legs in natural environments. *Didymuria violescens* (Leach), the spurlegged phasmatid, is an ideal species to explore this because it is a forest pest in many higher elevation areas of Victoria and New South Wales (Campbell and Hadlington 1967, Neuman *et al.* 1977, Schoenborn *et al.* 2003), and large samples are easily collected.

The goals of this field study were: (i), to sample a population from hatching to maturity to determine rates of autotomy and regeneration, and (ii), to explore if autotomy/regeneration affected survivorship.

Methods

An outbreak population of *D. violescens* was located in Bago State Forest, New South Wales, on 19 November, 2003 and studied until 6 February, 2004 (insects hatched in November and took ~3 months to mature). A survey of the area determined the outbreak to be approximately 50 m x 120 m (while some individuals were found outside this area, the majority of individuals were found within it). Within the 50 x 120 m plot, we set up 5 transects, each 120 m long and 8 m apart. Nymphs were collected along each transect. Each individual was measured for body length, examined for missing and/or regenerated legs, and released where it was collected. The measurements were used to ascribe each specimen to instar using the data of Neuman (1974) as follows: instar 1 = 1.80 cm (S.E. ± 0.5 cm), instar 2 = 2.88 cm (S.E. ± 0.5 cm), instar 3 = 3.89 cm (S.E. ± 0.6 cm), instar 4 = 5.74 cm (S.E. ± 0.8 cm), instar 5 = 7.67 cm (S.E. ± 0.8 cm), adult/instar 6 = 8.68 cm (S.E. ± 0.8 cm). Since the sexes are not distinguishable until the 3rd instar, rates of autotomy and regeneration for males and females were combined until maturity. After all transects were completed (approximately 10 days), we did not re-sample the area for another 5-10 days. This waiting period was sufficient to allow moulting to the next instar and minimized the likelihood of measuring the same individual in the same instar twice. The population was sampled during all instars except the fifth; these individuals can have completely regenerated legs that are only slightly shorter than non-regenerated legs, precluding scoring of regenerated legs in the field.

In addition to the data collected on the instar 1-4 nymphs, 803 adults (411 males, 392 females) were collected and brought back to the laboratory. We measured their body length and all six leg lengths. Because regenerated legs are always smaller than non-regenerated legs, paired t-tests between opposite legs (*e.g.* both front legs, both middle legs, and both hind legs) were used to identify regenerated legs.

To test if autotomy/regeneration affected survivorship, two 'expected' rates of regeneration were calculated. First, we calculated the minimum 'expected' rate of leg regeneration in adults by summing the rates of autotomy we observed in instars 2-4, since these individuals presumably commenced the regeneration process. Rates of autotomy for instar 1 individuals were not included in this 'expected rate' because they do not commence regeneration until instar 3; our proportion of instar 2 individuals missing one leg (13.2%) likely included many of the instar 1 individuals missing one leg (7.7%). Thus, if losing a leg had little or no effect on survival we expected at least 34.0% of sampled adults to have a partially or completely regenerated leg based on the observed rates of autotomy in instars 2-4 (13.2% instar 2 + 11.2% instar 3 +

9.6% instar 4 = 34.0%, see Table 1). Second, we calculated a minimum 'expected' rate of completely regenerated legs in adults by summing the rates of partially regenerated legs in instars 3 and 4, again because these individuals presumably completed the regeneration process (10.2% + 8.7% + 3.2% = 22.1%, see Table 1). Thus, if regenerating a leg has little or no effect on survival we expected to see at least 22.1% of sampled adults with completely regenerated legs. Although we did not sample instar 5, individuals that lose a leg at this instar do not regenerate it, and would therefore not contribute to the overall rates of partially or completely regenerated legs in adults.

Results and discussion

Didymuria violescens lost legs during all stages of development (Table 1). In each nymphal instar, approximately 10% of the individuals were missing one leg. A relatively high proportion of adults (15.3%) showed evidence of new leg losses, but this figure represents new loses during both instar 5 and adults. Approximately 25% of all sampled adults regenerated at least one leg during development (24.2% of males [n = 411]; 25.6% of females [n = 392]). 5.7% of adults had two regenerated legs and 3.2% had three or more (n = 803).
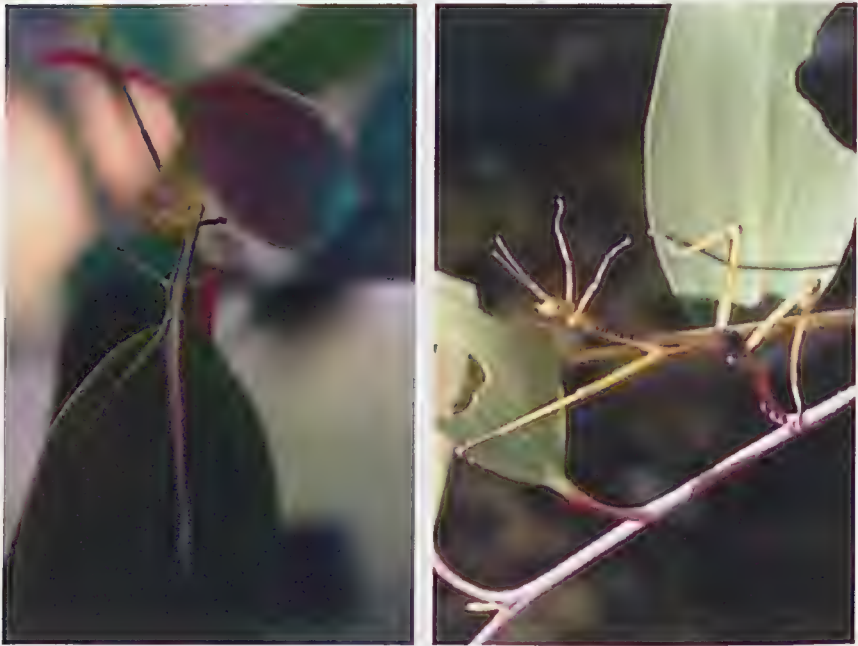
Autotomy and/or regeneration appeared to have negatively affected survivorship. Based on how often nymphs lose legs, we expected to see at least 34% of the adults in this population with evidence of regeneration but sampling revealed only 25.2% (9.4% of adults with partially regenerated legs + 14.8% of adults with completely regenerated legs). This pattern persisted when we used partial leg regeneration instead of autotomy to predict regeneration rates in adults. Based on the proportion of individuals with partially regenerated legs in instars 3 and 4, we expected to see at least 22.1% of adults with completely regenerated legs but sampling revealed 14.8%. Although we did not have data on the proportion of instar 5 individuals with partially or completely regenerated legs, it is likely the decreased survivorship patterns would persist and/or be strengthened by these data.

In addition, progression through instars generally showed a drop of a few percent in sequential regeneration. For example, 13.2% of instar 2 individuals were missing a leg and only 10.2% of instar 3 individuals had a first stage regenerated leg. Similarly, 11.2% of instar 3 individuals were missing a leg and only 8.7% of instar 4 individuals had a first stage regenerated leg. It is also noteworthy that only 3.2% of instar 4 individuals had a second stage regenerated leg, while 10.2% of instar 3 individuals had a first stage regenerated leg. This might suggest: (i), a sampling error or (ii), a disproportionately severe survivorship effect on instar 3 individuals with a first stage regenerated leg in this population.

All these patterns suggest decreased survivorship in individuals that are missing and/or in the process of regenerating legs. They are consistent with

Table 1. Frequencies of leg autotomy and regeneration in a population of *Didymuria violescens*.

Instar	Number (N)	% of N missing one leg	% of N in first stage of regeneration	% of N in second stage of regeneration	% of N with completely regenerated legs
1	482	7.7	-	-	-
2	502	13.2	-	-	-
3	509	11.2	10.2	-	-
4	519	9.6	8.7	3.2	-
5	Not sampled				
Adult	803	15.3	-	9.4	14.8

Fig. 1. Regenerated legs in *Didymuria violescens*: (left) an individual with a front right leg in the first stage of regeneration; (right) an individual with both front legs in the second stage of regeneration.

studies on crabs (Decapoda: Brachyura) that revealed a low percentage of adults with regenerated appendages compared with the high proportion of non-adults that were missing appendages (Savage *et al.* 1975, McVean and Findlay 1979, Lysenko *et al.* 2000). A mark-recapture study would further test the effects of autotomy and/or regeneration on survivorship.

Conclusion

Sampling *D. violescens* during their development revealed patterns about how often legs are autotomised and regenerated, as well as insight into its effects on survivorship. Recognizing that leg autotomy and regeneration occurred in approximately 25% of individuals in this population suggests that factors affecting autotomy and regeneration may be important agents of selection. More field research into other species will reveal if the rates of autotomy and/or regeneration in *D. violescens* are typical of other phasmids.

Acknowledgements

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